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Short communication

Long-lasting pain-related behaviors in mouse chronic cystitis model induced by a single intravesical injection of hydrogen peroxide

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ABSTRACT

We previously established a long-lasting cystitis model by an intravesical injection of hydrogen peroxide (H_2O_2) into mice. In this study, we assessed the pain-related behaviors in the cystitis model. An intravesical injection of 1.5% H_2O_2 transiently decreased spontaneous locomotor activity at 3 h after injection, indicative of acute spontaneous pain. In contrast, licking response to a bladder distention was slowly observed as licks to the lower abdomen at 7 and 14 days after injection, which was attenuated by amitriptyline and morphine, but not by oxybutynin. These results suggest that H_2O_2-induced chronic cystitis model shows delayed and long-lasting painful pathological condition.

Chronic inflammation in the urinary bladder generally causes urinary frequency, urgency, nocturia and pelvic or lower abdominal pain (1). Chronic inflammatory bladder diseases, such as interstitial cystitis/bladder pain syndrome (IC/BPS), are caused by infectious and noninfectious etiology. However, there are little or no reliably effective therapies and drugs for IC/BPS (2). For the current therapy to relieve BPS, tricyclic antidepressants, nonsteroidal antiinflammatory drugs (NSAIDs) and opioids are used for BPS (3, 4), although their analgesic efficacies are limited. BPS has been evaluated in a variety of experimental cystitis animal models, such as cyclophosphamide-induced cystitis model (5). However, since these models are characterized as acute inflammation, the durations of pain-related behaviors are usually short, which disagree with the pathology of chronic cystitis.

We recently established a cystitis mouse model induced by hydrogen peroxide (H_2O_2), which showed relatively long-lasting inflammatory and overactive bladder. An intravesical (i.ves.) injection of H_2O_2 increased the number of voids and bladder weight by 1 day and lasted up to 7 days after the injection, which was longer than that in existing cystitis models. In this model, initial H_2O_2-induced urothelial damage and hyperpermeability are considered to trigger long-lasting bladder inflammation (6). Thus, the H_2O_2-induced cystitis is a simple and useful model for chronic cystitis. However, pain-related behaviors in this model remained to be clarified. In cyclophosphamide-induced cystitis model mice, bladder pain-like spontaneous nociceptive behaviors, such as licking and biting to the lower abdomen close to the bladder, and referred hyperalgesia assessed by von Frey filaments (7). However, we could not detect such bladder pain-like behaviors in the H_2O_2-induced cystitis model mice (preliminary data). In this study, we examined two types of pain-related behaviors, including the reduced spontaneous locomotor activity and the bladder distension-evoked licks to the lower abdomen, in the H_2O_2-induced cystitis model mice.

This study was carried out in accordance with the recommendations in the Guiding Principles for the Care and Use of The Japanese Pharmacological Society. The protocol was approved by the Kyoto University Animal Research Committee. Female C57BL/6J mice aged between 5 and 6 weeks-old (Japan SLC, Shizuoka) were housed under constant ambient temperature (24 ± 1 °C) and light–dark cycle with feeding ad libitum.

H_2O_2-induced cystitis model was made as described previously (6). Briefly, under 2–3% isoflurane anesthesia, 50 μL of saline or 1.5% H_2O_2 saline solution was injected into the bladder through a

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catheter. The H_2O_2 solution was drained from the bladder after 30 min.

For the measurement of locomotor activity, mice were allowed to habituate to the experimental condition in an individual clear plastic cage (10 cm W × 20 cm L × 30 cm H) for 30 min, and the behaviors were videotaped for 15 min. Move distance, average speed of movement, and freezing time were analyzed using ANY-maze™ (Stoelting Co., USA).

For the measurement of bladder distension-evoked pain-related behavior, mice were habituated to the experimental condition in the plastic cage for 30 min. Under 2–3% isoflurane anesthesia, 50 μL of saline was injected into the bladder through a catheter. Mice were kept for 1 min under 1% isoflurane anesthesia, and the behaviors were videotaped under fresh air condition. After the recovery from anesthesia, the time spent in licking of the lower abdomen was measured for 20 min. To examine the effects of drugs, oxybutynin chloride, indomethacin (Sigma–Aldrich Japan, Tokyo), amitriptyline hydrochloride (LKT Laboratories Inc., USA) or morphine hydrochloride (Takeda Pharmaceutical Co., Osaka) was freshly dissolved in a saline containing 5% DMSO and 2% Tween80. One of these drugs was administered intraperitoneally (i.p.) 30 min before the test. The doses of drugs were chosen so that they inhibit frequent urination in the H_2O_2-induced cystitis model (6).

All data are presented as the mean ± S.E.M. Statistical significance was calculated by two-way analysis of variance (ANOVA), or two-way ANOVA for repeated measures, followed by Bonferroni *post hoc* test.

Reduced locomotor activity in rodents is proposed as spontaneous visceral pain-related behavior, as previously reported in cyclophosphamide-induced cystitis model mice (7). In this study, we examined the effect of i.ves. injection of H_2O_2 on the spontaneous locomotor activity (Fig. 1). An i.ves. injection of 1.5% H_2O_2 significantly increased the freezing time ($F_{1,40} = 13.84$, $P < 0.001$), and decreased the move distance ($F_{1,40} = 6.26$, $P < 0.05$) and average speed ($F_{1,40} = 5.67$, $P < 0.05$), although i.ves. saline injection had no effect. The significant differences were observed only at 3 h (freezing time, move distance and average speed) and 1 day (move distance) after H_2O_2 injection. They recovered gradually from the next day and to the control level 7 days after the injection.

To assess the delayed phase of pain-related behaviors in the H_2O_2-induced cystitis model, the effect of bladder distension was examined. An i.ves. injection of 50 μL of saline evoked licking of the lower abdomen. The time spent in licking was significantly increased in H_2O_2-induced cystitis model mice ($F_{1,57} = 12.17$, $P < 0.001$). The significant increases were observed at 7 and 14 days, but not at 1 day, after H_2O_2 injection, compared with that in control mice (Fig. 2A).

We examined the effects of therapeutic drugs on the bladder distension-evoked licking 7 days after H_2O_2 injection (Fig. 2B). Amitriptyline (1 mg/kg) or morphine (3 mg/kg) significantly attenuated the increased licking time, compared with the i.p. vehicle-administered group. Indomethacin (3 mg/kg) tended to, but not significantly, attenuate the increased licking time, while oxybutynin (3 mg/kg) had no effect. In i.ves. saline-injected control mice, each drug had no significant effect on the licking time, although oxybutynin and indomethacin slightly tended to increase it.

In this study, we assessed two types of pain-related behaviors in the H_2O_2-induced cystitis model mice. The decreased locomotor activity is consistent with the previous report in cyclophosphamide-induced cystitis model (8). However, the hypolocomotion was observed only within 1 day after the H_2O_2 injection, suggesting acute pain-related behavior. In the acute phase of this model, H_2O_2 destroyed bladder urothelium leading to severe acute inflammation accompanied with bladder urothelial

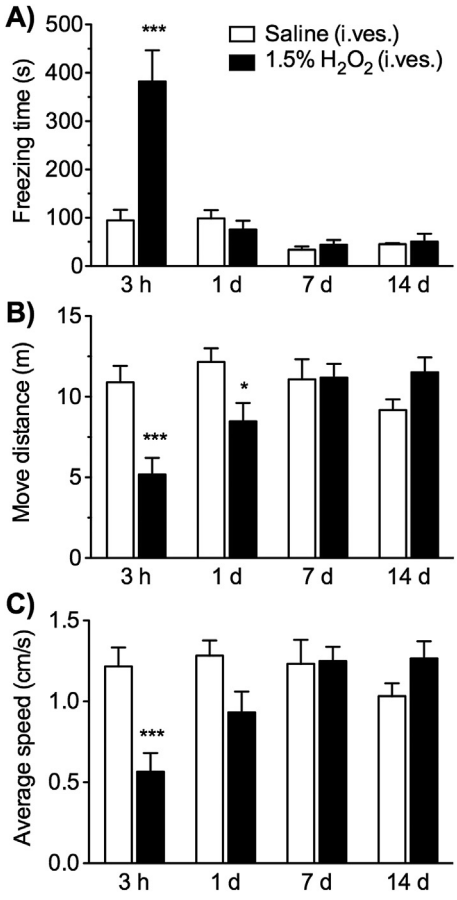

Fig. 1. Effects of an intravesical injection of H_2O_2 on the spontaneous locomotor activities. Mice were injected intravesically (i.ves.) with saline or 1.5% H_2O_2. Three hours, 1, 7 and 14 days after the injection, freezing time (A), move distance (B) and average speed (C) were measured for 15 min $n = 6$. $^*P < 0.05$, $^{***}P < 0.001$, compared with i.ves. saline-injected control mice.

and vascular hyperpermeabilities, edematous thickening in the lamina propria, infiltration of inflammatory cells, and production of inflammatory cytokines (6). These severe damages in the bladder may immediately evoke the hypolocomotion, and recover with the restoration of bladder tissue within several days. Furthermore, we could not detect refereed hyperalgesia.

By contrast, bladder distension-evoked licking was observed in the delayed phase of H_2O_2-induced cystitis model, which was attenuated by analgesics, suggesting delayed and long-lasting pain-related behavior. Consistent with the findings that, anticholinergic drugs alleviate urinary and bladder dysfunction (9), oxybutynin reduces the urinary frequency in this model (6), but shows neither anti-inflammatory nor analgesic effect. Unlike other cystitis models, following the recovery of bladder damages, bladder inflammation is prolonged, and the remodeling and hyperplasia of the bladder urothelium occur (6). Inflammatory cytokines induce the hypersensitivity of the bladder sensory nerves leading to bladder overactivity (10). Thus, it is suggested that hyperplastic urothelium following the recovery of bladder damages and hypersensitivity of the bladder sensory nerves may contribute to the delayed and long-lasting pain-related behavior. On the other hand, prostaglandins are released immediately after synthesis from the urothelium of overactive bladder models in response to the bladder distension (11). However, the present results that the analgesic effect of an NSAID was weak suggest that the pain-related behavior is not merely mediated through prostaglandin synthesis and

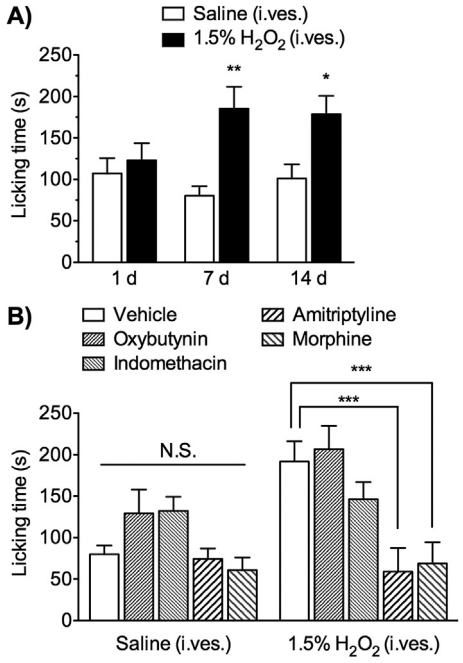

Fig. 2. Bladder distension-evoked licking to lower abdomen in H_2O_2-induced cystitis model. (A) Mice were injected intravesically (i.ves.) with saline or 1.5% H_2O_2. 1, 7 and 14 days after the injection, mice were injected with 50 μL of saline to dilate bladder. After the recovery from anesthesia, the time spent licking of the lower abdomen was measured for 20 min $n = 6–15$. $^*P < 0.05$, $^{**}P < 0.01$, compared with i.ves. saline-injected control mice. (B) Effects of therapeutic drugs on the increased total licking time in H_2O_2-induced cystitis model mice. Seven days after the i.ves. saline or 1.5% H_2O_2 injection, oxybutynin (3 mg/kg), indomethacin (3 mg/kg), amitriptyline (1 mg/kg), morphine (3 mg/kg) or vehicle was given intraperitoneally 30 min before testing. The licking time was measured for 20 min $n = 7–13$. $^{***}P < 0.001$, N.S. = not significant.

bladder inflammation, consistent with minimal evidence of chronic inflammation in non-ulcer type IC/BPS patients (12). It is possible that initial severe painful stimuli and chronic bladder inflammation might lead to the central sensitization of pain pathway in the spinal cord and brain, resulting the delayed and long-lasting bladder pain (13). It is noted that the licking of lower abdomen by i.ves. saline injection may represent urethra pain rather than bladder pain, as previously noted (14).

In conclusion, the present study revealed that H_2O_2-induced chronic cystitis model shows delayed and long-lasting painful pathological condition. This model may help to study chronic bladder pain in chronic cystitis such as IC/BPS.

Conflict of interest

The authors indicated no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2015.11.003.

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